HLA epitopes in kidney transplantation: from basic science to clinical application

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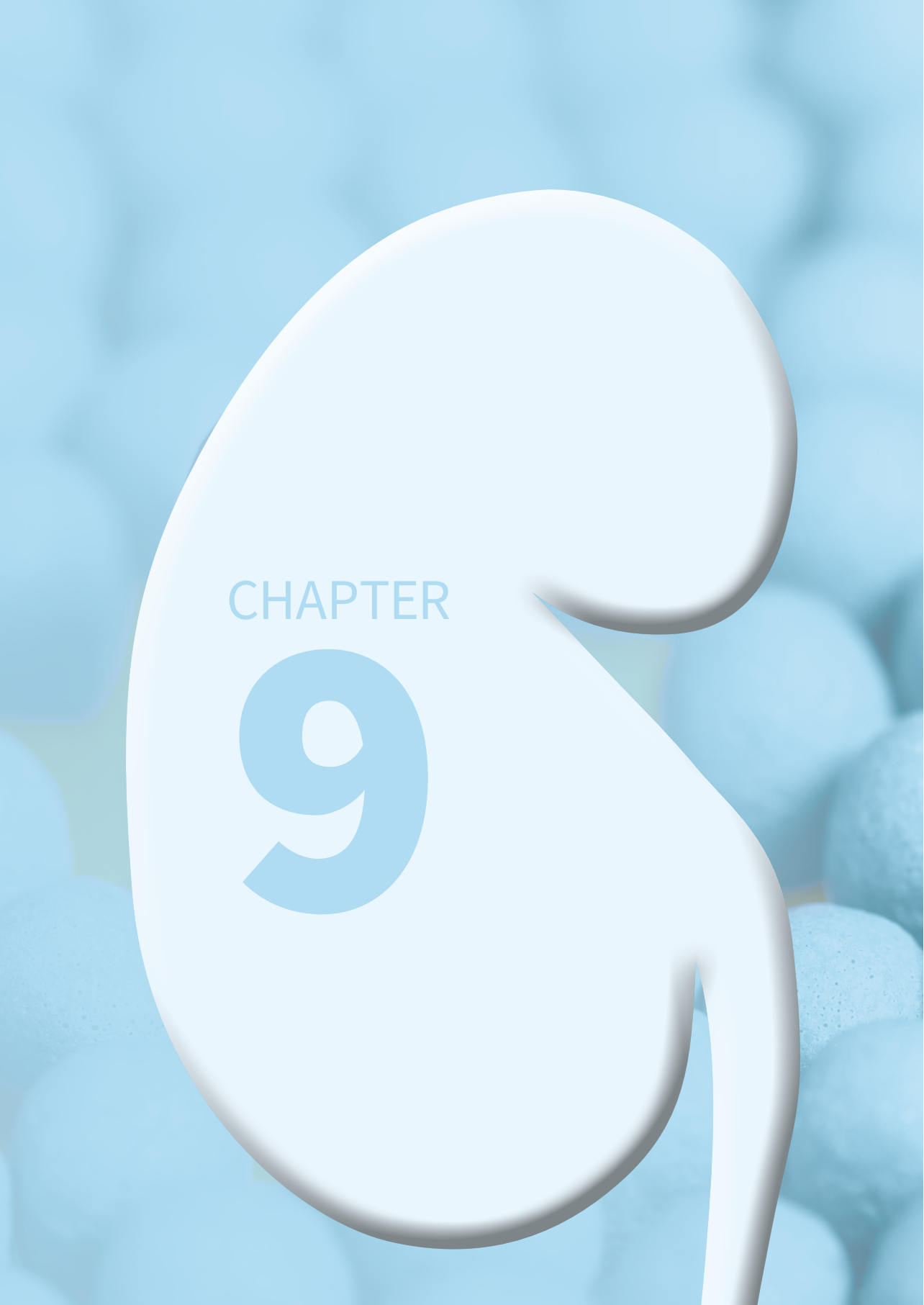
CHAPTER
9

General Discussion and Future Perspectives

HLA MATCHING IN KIDNEY TRANSPLANTATION

After the discovery of the Human Leukocyte Antigen (HLA) system in the 1950s, Paul Terasaki demonstrated the correlation between HLA matching and kidney allograft survival in 1966[1]. Since then, HLA matching has been one of the cornerstones of transplantation and transplant organizations across the world have included HLA matching in their algorithms for organ allocation. However, with the introduction of modern immunosuppressive agents, the priority of HLA matching has decreased and questions have been raised about the importance of HLA matching in kidney transplantation[2]. Still, HLA mismatching remains an independent predictor for graft loss[3-5], and with the introduction of the HLA epitope paradigm[6], a new way of HLA compatibility analysis has emerged. Since the first description of HLA epitopes, epitope based matching in transplantation has been broadly discussed in literature[7-9]. Various methods have been developed for HLA epitope analysis, but HLA eplets, as described in the HLA Eplet Registry and incorporated in HLAMatchmaker[10], remain most well-known. However, the actual practical implementation and feasibility of eplet matching has remained unclear, and to date only one study has prospectively investigated eplet matching in kidney transplantation[11]. In fact, due to the lack of empirical evidence for clinically relevant eplets, the application of eplet-matching in transplantation has even been deemed premature and the question has been raised whether eplet matching will actually reduce the complexity of HLA matching[12]. In this chapter the various applications of eplet-matching in transplantation will be discussed, including 1) organ allocation in deceased donor programs, 2) living donor selection, 3) to increase in transplantability of highly immunized patients and 4) post-transplant risk stratification to facility personalized immunosuppressive management, along with the challenges and gaps in current knowledge regarding these approaches.

Identification of immunogenic eplets is required for clinical application of HLA epitopes

The lack of empirical evidence for clinically relevant eplets is the one of the main obstacles for implementation of eplet-matching in transplantation. As the eplet repertoire has been theoretically defined based on HLA amino acid sequences and not on proven immunogenicity, the question remains which eplet mismatches are immunogenic and which are permissible[12, 13]. The identification of clinically relevant eplets is a crucial condition for the modification of allocation algorithms towards eplet based matching, as it would be unacceptable that patients might be denied an organ offer based on eplet mismatches which are not proven immunogenic.

As discussed in **Chapter 2**, antibody verification is required to validate that eplets can actually be bound by antibodies. Much experimental evidence has been gathered over the years, which is summarized in The HLA Eplet Registry, an online database of HLA eplet data[14-16]. Our review of The HLA Eplet Registry in **Chapter 4** provided insight in the different methods that have been used for antibody verification, and showed that not all eplets considered antibody-verified by The HLA Eplet Registry, were verified based on high quality, peer reviewed research. Furthermore, it elucidated that especially for HLA class II, there are several theoretical eplets

for which no antibody verification has been performed yet. Accordingly, we generated several human HLA-DQ-specific recombinant monoclonal antibodies (mAbs) by single cell sorting HLA-specific memory B cells from pregnancy-immunized women, as demonstrated in **Chapter 3.** These mAbs are not only excellent tools for the antibody verification of HLA-DQ eplets, but they could also be utilized for further in-depth investigation of the epitope-antibody interaction. One of the exciting next steps in the process of further understanding the fundamental biology of alloantibodies, is the actual visualization of the antibody binding to its target HLA. Recently, the first crystal structure of a HLA-A*11:01-specific antibody bound to its target HLA has been reported[17]. The characterization of the epitope-paratope interaction demonstrated that the amino acid that was predicted to be crucial for antibody binding, was indeed part of the epitope. However, unlike the HLA-specific antibodies generated in our laboratory, the described HLA-A*11:01-specific antibody was generated using a phage library, and may not represent an antibody developing during a human immune response. Currently, studies are ongoing to characterize the binding of fully human HLA-DR and -DQ-specific mAbs using cryogenic electron microscopy, which allows for visualization of the binding of these mAbs to their target molecules. Another approach to provide more insight in which amino acids are crucial for binding, is by mutagenesis of HLA molecules as described in **Chapter 5**. Moreover, these approaches will also increase our understanding of the electrostatic and physiochemical properties of amino acids that are relevant for immunogenicity[18-20]. Ultimately, since antibody verification of eplets using human mAbs is a slow and laborious process, ideally a prediction algorithm of HLA epitope immunogenicity should be developed. Preferably, this algorithm would incorporate all existing evidence generated by the reactivity patterns of HLA-specific mAbs, the identified crucial amino acids by mutagenesis and crystallography or cryo-EM, and the physiochemical and electrostatic properties of the amino acids involved. Additionally, also the role of T cell epitopes has to be investigated further, as T cell help is required for the initiation of a long-lived antibody response[21]. Although predicted T cell epitopes have been associated with graft failure in kidney transplantation[22], there is no experimental evidence for which peptides derived from allogeneic HLA are generated in the lysosomal compartment and which peptides are actually presented by HLA class II molecules *in vivo.* The approach of predicting HLA-derived T cell epitopes presented in recipient class II molecules is at the moment merely based on the underlying amino acid differences between HLA alleles and relatively low peptide binding affinity, and is therefore not inherently different than the algorithms for B cell epitope prediction, albeit with a lower specificity. Lastly, although eplets have been the basis for the majority of HLA molecular mismatch studies, it is important to note that even one single amino acid mismatch can induce antibody formation[23-25]. Therefore, it is possible that only one amino acid residue, instead of the amino acid configuration that comprises an eplet, is essential for antibody induction. Indeed, several eplets in the HLA Eplet Registry consisting of several amino acids have in time been reclassified into eplets consisting of a single amino acid. This is one of the reasons behind the development of HLA-EMMA, a software program that calculates amino acid mismatches of mismatched HLA between donor and recipient[26].

Further steps needed for implementation of epitope matching in clinical practice

Besides the gap in knowledge of the antibody-epitope interaction, there are several other obstacles on the road towards implementation of epitope matching in transplantation. Firstly, in order to perform HLA matching on the epitope or eplet level, high-resolution HLA typing of the donor and potential recipient(s) is required. As described in **Chapter 7** and **Chapter 8**, second-field typing for donor-recipient pairs was performed retrospectively in most cases, since this was not routinely performed at the time of living donor transplantation. In the meantime, many transplant centers have introduced high resolution typing for the living donor transplantation setting. In contrast, high-resolution typing is not yet routinely performed for deceased donor transplantation in most transplant centers, as HLA typing on the second field level is not only more costly than low-resolution typing, but also takes more time to complete. Most commercial kits offering high resolution typing based on next generation sequencing take 1 to 5 days for completion[27], making it an unsuitable technique for the typing of deceased donors. Nonetheless, a recent study described the development of a high resolution typing method using Nanopore sequencing, which resulted in high-resolution typing for 11 loci within 4.5 hours, indicating that second field typing for deceased donors is within reach[28]. Several commercial companies are currently optimizing the Nanopore sequencing workflow for deceased donor typing.

A provisional solution for the lack of high resolution typing is imputation of second field typing based on low resolution haplotypes[29]. This method has been applied frequently in large cohort studies investigating eplet association with transplant outcomes. However, as this method can lead to inaccuracies in eplet compatibility estimations, it is not suitable for the clinical setting[30].

Secondly, as HLA allele frequencies vary considerably amongst different populations in the world[31], this also means that eplet frequencies will significantly differ across different populations. This has to be taken into account in studies that investigate differential immunogenicity of individual eplets, because a very high or very low frequency of an eplet in a population can skew immunogenicity scores, which may consequently not be applicable in other populations[24]. The fact that this issue needs consideration is illustrated by the situation in the United States, where it became clear that African Americans were disadvantaged regarding access to kidney transplantation due to HLA matching requirements[32]. Subsequently, priority for HLA-A and HLA-B matching was eliminated in the kidney allocation system of the United Network of Organ Sharing[33,34]. Although Dutch law does not allow recording of ethnicity, it can be assumed that the populations described in **Chapter 6**, **Chapter 7** and **Chapter 8** were predominantly Caucasian. Hence, data regarding eplet frequencies in different populations are required, so that the consequences for the implementation of eplet-based allocation algorithms in ethnically diverse populations can be investigated.

Related to the issue of equity is the concern that epitope based matching would lead to longer waiting times on the transplant waiting lists, especially for ethnic minorities in diverse popula-

tions. As quality of life is poor for patients on dialysis, and the survival benefit of transplantation as compared with dialysis is signficant[35], it would not be acceptable that better HLA compatibility by utilizing epitope matching would be at the expense of longer waiting times. Currently there are not sufficient data about the consequences of epitope matching for the kidney transplant waiting lists. A simulation study in British Columbia, Canada suggested that eplet matching would not only reduce HLA complexity and would minimize the consequences of ethnic diversity, but would be feasible within a waiting list of 250 patients[36]. However, simulations regarding waiting times were not performed. Another study performed an allocation simulation to study the effect of T cell epitope matching on waiting times in the Eurotransplant region[37] and found that this approach did not significantly impact waiting times. However, since no specific attention to ethnic minorities has been given, more data are required to ethically justify changing matching algorithms.

Epitope analysis in high sensitized patients

Although the road to implementation of epitope matching in deceased donor allocation algorithms still seems long, there is already a group of transplant patients that can benefit from epitope analysis. In highly sensitized patients, epitope knowledge can be utilized to increase the chance of finding a suitable donor. This application of epitopes was already described early 2000s by Rene Duquesnoy and Frans Claas[38, 39] and was in fact the primary concept behind HLAMatchmaker. In 2019, 5.6% of patients on the Eurotransplant waiting list was highly sensitized[40]. The chance of finding a suitable donor for these patients is very slim due to the large number of unacceptable antigens. Epitope analysis can determine which of the unacceptable antigens as determined in a single antigen bead assay can be explained by a previous immunizing event, and are therefore truly unacceptable. If the reactivity cannot be explained by an immunizing event or a shared epitope/eplet thereof, this bead reactivity might be background or non-specific binding, and this allele should not be listed as unacceptable, but rather be considered as a risk factor, taking into account MFI value of the reactive allele. A recent study from Portugal showed that calculation of an eplet-based virtual PRA increased the transplant probability for highly-sensitized patients[41] and in the Eurotransplant region, eplet analysis is used for patient acceptance in the Acceptable Mismatch Program for highly sensitized patients[42]. In the Eurotransplant Acceptable Mismatch Program only antibody verified eplets as described in **Chapter 4** are considered. Additionally, eplets present on antigens towards which no antibodies have formed can be extrapolated to antigens not tested in single antigen bead assays, to maximize the number of acceptable antigens defined[38]. Therefore, eplet analysis in these specific group of patients is very valuable and single antigen bead vendors are developing their software to enable eplet analysis for serum reactivity patterns.

Living donation

While the previous section was predominantly related to deceased donor transplantation, determining the level of epitope mismatches can also be of value in living donor transplantation. Primarily in patients that are likely to require a re-transplantation later in life, it is critical to limit the formation of donor-specific antibodies (DSA) formation at the first transplantation,

so that there are no pre-existent DSA that can impede a re-transplantation at a later timepoint. As discussed in **Chapter 2**, HLA compatibility analysis on the epitope level is far more detailed than on the antigen level. In cases where there are multiple potential donors who all have a certain number of antigen mismatches with the recipient, epitope analysis can indicate which donor has the least epitope mismatches and thus has the lowest chance of harboring immunogenic epitopes (Figure 1). Currently, there has not been a study in which this approach has been investigated methodically. Therefore, in order for epitope or eplet analysis to be routinely used in living donor transplant selection, HLA lab directors should be educated in HLA epitope analysis and this approach should be further investigated in clinical studies. Regardless, other factors besides HLA will contribute to the definitive selection of a living donor, including age, medical history, and psychological and social factors.

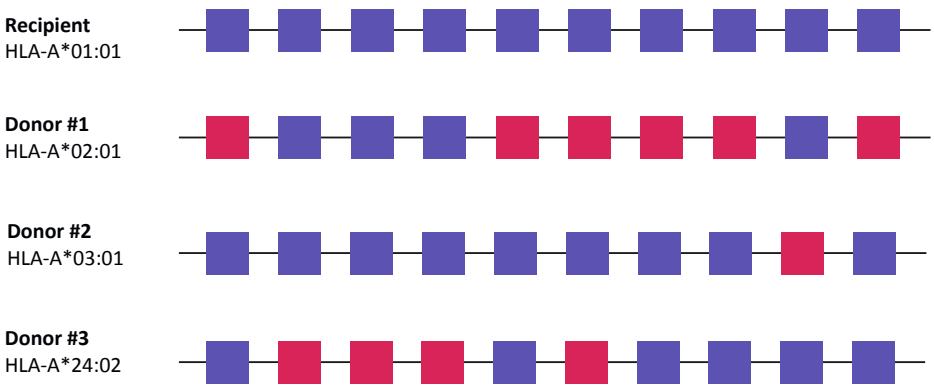

Figure 1. Living donor selection based on eplet mismatches. Every potential donor has a single HLA antigen mismatch with the recipient, but a different number of antibody-verified eplet mismatches. In this case, donor #2 would be the best option for the recipient, as there is only one eplet mismatch with the recipient. Squares: antibody-verified eplets as defined in **Chapter 4**. Eplet mismatches are assessed taking into account the full HLA typing of the recipient. Blue, matched eplets; red, mismatched eplets.

Eplet mismatch levels for post-transplant risk stratification

The identification of immunogenic and permissible epitope or eplet mismatches is essential for the implementation of epitope matching in deceased donor allocation schemes and epitope analysis for living donor selection. However, deciphering immunogenicity of individual eplet mismatches may be less relevant for utilizing eplet mismatch levels for post-transplant risk stratification, as eplet mismatches would be merely used as a tool to assess the risk for immunological rejection after transplantation, instead of affecting organ allocation and donor selection. Many studies have demonstrated that eplet mismatch loads are associated with the risk of DSA formation, rejection and graft loss after transplantation[43-48]. Furthermore, Wiebe *et al*. have shown that HLA class II eplet mismatch load was associated with the tacrolimus through levels that are required to prevent DSA formation[49]. As there still is an unmet need for tools that could guide personalized immunosuppressive therapy in transplantation[50,51], HLA epitopes in the form

of amino acids or eplets, could be used as a parameter in post-transplant risk stratification. However, there are several challenges considering the application of epitopes for this purpose.

Firstly, it is unclear which HLA loci should be considered in post-transplant risk stratification. The majority of studies have reported data on HLA class II, as we have also described in **Chapter 6** and **Chapter 7**. However, it is not clear if only HLA class II, or even only HLA-DQ should be considered, or that all HLA loci should be taken into account. Secondly, there are several different ways of calculating HLA epitope mismatch loads (as described in **Chapter 2**), which all have been associated with transplant outcomes like DSA formation and graft survival. The use of different computer programs, including HLAMatchmaker, the Electrostatic Mismatch Score (EMS) and HLA-EMMA, makes it difficult to compare studies, because each method will result in different optimal cutoff values and ranges. This heterogeneity impedes the ability to draw conclusions that can lead to cutoff values that can be validated in other cohorts. Lastly, the definition of a cutoff value that divides a study population between low risk and high risk, results in the possibility that when this cutoff will be applied in a general population, a patient who received a graft bearing an eplet mismatch level below the cutoff still received an organ containing a highly immunogenic eplet mismatch that could lead to DSA formation. Additionally, optimal cutoff values for risk stratification presumably will be population-specific. Hence, before guidelines can be developed that can be implemented in clinical practice, it is necessary that there is consensus in the field regarding the way forward. A possible strategy would be to take a step back and first focus on amino acid mismatch analysis, because this type of analysis is consistent and not subject to change like eplet definitions are. One of the other advantages of amino acid mismatch analysis is that there are multiple analysis methods and software programs available, so that investigators can use the program which they are familiar with, while the analyses remain uniform and comparable. Once cutoff values have been defined for a certain population, they should be validated in other cohorts. Although the amino mismatch analysis as described in **Chapter 6** suggested that there is a linear effect on DSA formation and graft loss, cutoff values would be still required in clinical practice to classify patients as low and high immunological risk.

In order to take post-transplant risk stratification based on epitopes forward, studies are required to investigate whether epitope mismatch load can identify patients that can benefit of reduction of immunosuppression. In the retrospective analysis of the CTOT-09 study, HLA-DQ eplets were associated with DSA formation after tacrolimus withdrawal[52] and in the CELIMINN trial, HLA class I and HLA-DQ eplets predicted *de novo* DSA formation[53]. Also in our analysis of mesenchymal stromal cell (MSC) therapy and tacrolimus withdrawal described in **Chapter 7**, HLA-DQ eplets were associated with *de novo* DSA formation. A next step in the investigation of eplet mismatches for risk stratification is a prospective study that will randomize patients in one of two groups. In the first group, epitope mismatch analysis (either based on eplets or amino acids) will first have to be performed to categorize the patient as low or high risk. In case of low risk, there will be a predefined reduction of immunosuppression at a specific timepoint, such as lower tacrolimus though levels or complete withdrawal of a immunosuppressive drug. If the

patient is considered to be high risk, no minimization of immunosuppression will be performed. In the second group, epitope mismatch analysis is not performed *a priori* but only in *post-hoc* analysis, and patients are treated with the standard of care. Primary and secondary outcomes should include *de novo* DSA formation, rejection, graft loss, and adverse effects of immunosuppression such as infections and malignancies, measured during a follow-up period of at least five years.

Although previous tacrolimus withdrawal trials selected ''immune-quiescent'' or long term stable kidney transplant patients[52, 54], HLA compatibility analysis was not part of the method to select low risk patients. Epitope mismatch load could be used as an strategy to select low risk patients for inclusion of immunosuppressive weaning trials, but also in studies investigating cellular therapy with MSC. As the field of MSC therapy is advancing, there is increased attention for the use of allogeneic MSC therapy as opposed to autologous MSC. While concerns have been raised regarding the immunogenicity of MSC, especially when the MSC donor shares epitope mismatches with the kidney donor[55-57], we demonstrated in **Chapter 8** that shared amino acid mismatches were not associated with DSA formation in two cohorts of kidney transplant patients treated with allogeneic MSC. Future studies should validate these findings in different cohorts and should further explore the potential of using epitope analysis in donor selection for cellular therapy in transplantation.

Lastly, as opposed to identifying low risk patients for immunosuppression weaning trials, epitope mismatch load could also be used to select high risk patients for studies with rare endpoints, such as antibody-mediated rejection. Currently, as the incidence for antibody-mediated rejection is low, clinical studies investigating this outcome need to include very large numbers of patients to generate enough power for conclusive results. By selecting patients based on epitope mismatch load, the study population for intervention studies could be enriched for high risk patients, which would facilitate smaller study cohorts[58].

Conclusion

The clinical application of HLA epitopes in transplantation is promising and has several different approaches. However, it is of importance that the fundamentals of alloantibody biology and epitopes keep being studied, to unravel the factors that affect the relative immunogenicity of HLA mismatches. Excellent quality research in this field requires further development of human HLA-specific monoclonal antibodies, the collection of large datasets with high resolution HLA typed transplant recipients and donors, the availability of single antigen bead data for DSA analysis and kidney biopsy data for rejection. Furthermore, the time has come for the initiation of prospective studies that investigate the value of HLA epitope mismatch analysis in post-transplant risk stratification for reduction of immunosuppression.

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